Rec'd PCT/PTO 14 APR 2005

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PCT/EP03/11329

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Patentanmeldung Nr.
Patent application no.
Demande de brevet n° PCT/EP 03/04851

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Blatt 2 der Bescheinigung
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Page 2 de l'attestation

Anmeldung Nr.:
Application no.:
Demande n°: PCT/EP 03/04851

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Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention: ANIMAL MODEL TO EVALUATE VISCERAL PAIN PERCEPTION

Anmeldetag:
Date of filing:
Date de dépôt: 07 May 2003 (07.05.2003)

In Anspruch genommene Priorität(en)
Priority(ies) claimed
Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Benennung von Vertragsstaaten : Siehe Formblatt PCT/RO/101 (beigefügt)
Designation of contracting states : See Form PCT/RO/101 (enclosed)
Désignation d'états contractants : Voir Formulaire PCT/RO/101 (ci-joint)

Bemerkungen:
Remarks:
Remarques:

PCT REQUEST

PRD2004i

Original (for SUBMISSION) - printed on 06.05.2003 02:00:34 PM

IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	common representative
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V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	EP: AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT SE SI SK TR and any other State which is a Contracting State of the European Patent Convention and of the PCT, Ro^ *Ro/EP
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	US
VI-1	**Priority claim of earlier international application**	
VI-1-1	Filing date	15 October 2002 (15.10.2002)
VI-1-2	Number	PCT/EP02/11772
VI-1-3	PCT receiving Office	EP
VI-2	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)
VII-2	**Request to use results of earlier search; reference to that search**	
VII-2-1	Date	15 October 2002 (15.10.2002)
VII-2-2	Number	PCT/EP02/11772
VII-2-3	Country (or regional Office)	EP

ANIMAL MODEL TO EVALUATE VISCERAL PAIN PERCEPTION

This invention relates to an animal model to measure visceral pain by means of a balloon catheter and an implantable sensor module having transcutaneous telemetring ability. The implantable sensor module according to the invention is set up to receive both visceromotor and pseudoaffective responses of the test animal. In particular, this invention provides a non-human animal model wherein balloon catheter is an implantable balloon catheter, preferably implanted in the duodenum of the test animal and the implantable sensor is set up to receive input signals form at least one bipolar electrode pair and at least one blood catheter. In particular this bipolar electrode is set up to receive visceromotor responses, especially electromyography of the abdominal muscle and the blood catheter set up to register mean arterial pressure and heart rate of the abdominal aorta.

It is thus a further object of the present invention to provide a method for producing said animal as well as kits comprising a balloon catheter and an implantable sensor module for use in a method for producing said animals.

BACKGROUND OF THE INVENTION

The International Association for the Study of Pain has defined pain in the following way: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Mertz 1979)". The problem, however, is that pain cannot be measured directly in animals, but can only be estimated by examining their responses to nociceptive stimuli. Most models of nociception are based on behavioral responses to pain, ranging from the most elementary motor reflexes to far more integrated behaviors (escape, avoidance). A further problem with abdominal pain is that it is characterized by poor localization, abdominal cramps (visceromotor response) and autonomic (pseudoaffective) responses, including changes in respiration, heart rate (HR) and mean arterial pressure (MAP) that are difficult to score in a quantifiable and reproducible way.

The algesic writhing model has been used most commonly to study visceral pain in animals (Reichert, Daughters et al. 2001). In this model, an algesic solution is injected intraperitoneally into an awake animal and the number of writhes (stretches of the torso, hyperextension of the hind limbs with concave arching of the back and abdominal contractions) is scored. Due to ethical constraints, repeated assessments in a single animal cannot be performed, thereby compounding the difficulty of assessing tolerance development to analgesic agents. Furthermore, this model lacks escapability, specificity and is not related to human pathology. Mechanical induced stimuli of the viscera (distention of hollow viscera) reproduce a natural visceral stimulus, which mimics more closely visceral pain in humans, and is found aversive (avoidance/writhing behavior) in animals (Gebhart and Ness 1991;Ness, Randich et al. 1991;Ozaki, Bielefeldt et al. 2002). These visceral pain models produce quantifiable pseudoaffective reflexes, which include an increase in MAP and HR in the awake animal (Danzebrink and Gebhart 1990;Danzebrink and Gebhart 1991), although these are attenuated or even reversed by certain anesthetics (Ness and Gebhart 1988;Diop, Riviere et al. 1994;Ness 1999).

Colburn and colleagues (1989) studied the visceromotor response to volume-fixed duodenal distention in conscious, freely moving rats by scoring behavioural responses to pain such as shaking, exploring, grooming abdominal region, stretching or immobility and by scoring the occurrence of abdominal contractions. They demonstrated a graduated relationship between distending volume and the frequency of abdominal cramps. Furthermore, they showed that the visceromotor response to duodenal distention was inhibited by morphine in a dose-dependent manner.

In order to have a more quantifiable and reproducible model to study the visceromotor response to mechanical distention, several research groups are recording abdominal electromyography (EMG). Mostly, electrode wires were inserted into the abdominal or neck musculature and were exteriorized on the back of the animal from where it could be connected to an ink-writer or computer for EMG recording. These studies are done in the conscious *restrained* (Friedrich and Gebhart 2000;Ozaki, Bielefeldt, Sengupta, and Gebhart 2002;Bradesi, Eutamene et

~~al. 2002) or lightly *anaesthetized* (Ness, Lewis-Sides et al. 2001)~~ rat, to prevent damage of the exteriorized part of the balloon catheter and electrode leads due to biting and/or to minimalize background EMG noise induced by additional body movements (like exploration, grooming). However, in these studies both visceromotor and pseudoaffective responses to visceral pain **are affected** by the presence of anesthesia (Ness and Gebhart 1988;Ness 1999) and/or (restraint/handling) stress (Coutinho, Plotsky et al. 2002).

It would accordingly be interesting to have an animal model of abdominal nociception to analyze analgesic properties of new pharmaceutical compounds, wherein the usefulness of such a visceral pain model is determined by the following criteria (Ness and Gebhart 1990;Gebhart and Sengupta 1996): (1) the stimulus should reproduce as much as possible a natural stimulus and must produce pain in humans, (2) the stimulus must induce aversive animal behavior (escape, withdrawal, avoidance), (3) the stimulus must evoke pseudoaffective responses consistent with those in humans in response to visceral pain, (4) responses to the stimulus must be modulated by antinociceptive manipulations, (5) the responses should be quantifiable and reproducible and (6) the model should be as non-invasive as possible and able to be used in anaesthetized animals.

All of the models described above only partially meet these requirements. It is thus an object of the present invention to provide a new animal model to study visceral pain especially characterized in that it can be used for both acute and chronic analysis of analgesic properties and that it allows simultaneous and continuous measurement of both the visceromotor (abdominal EMGs) and pseudoaffective response (MAP and HR) in a conscious, *freely moving* animal.

The present invention solves this problem by using a chronically implanted balloon catheter in the duodenum to deliver duodenal distention and a chronically implanted transmitter connected to a bipolar electrode pair and blood catheter for **simultaneous** and **continuous** telemetric measurements of the visceromotor (abdominal EMGs) and pseudoaffective response (MAP and HR) respectively.

SUMMARY OF THE INVENTION

This invention relates to an animal model to measure visceral pain by means of a balloon catheter and an implantable sensor module having transcutaneous telemetring ability. The implantable sensor is capable to transmit data relevant to visceral pain to an apparatus outside the body capable of continuously monitoring the user's status and is capable to accept a plurality of input signals either simultaneously or sequentially, preferably from bipolar electrode pairs and blood catheters. As such, this model provides an adequate tool to measure visceromotor and pseudoaffective responses to visceral pain continuously and simultaneously in a non-human animal.

In a further embodiment this invention provides kits comprising a balloon catheter, an implantable telemetric sensor module, a bipolar electrode pair and a blood catheter, for use in a method to produce an animal model to measure visceral pain.

It is thus a further object of the present invention to provide a method for producing an animal model to measure visceral pain comprising; implanting a balloon catheter in the duodenum of said animal; and implanting a telemetric sensor module in the abdominal cavity of said animal wherein said telemetric sensor module is set up to receive input signals from at least one bipolar electrode pair and at least one blood catheter.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1. Drawing of the intra-gastroduodenal part of the silicone balloon catheter in uninflated and inflated condition.

Figure 2. Changes in gross activity (counts/min) induced by staircase increases in distention volume (ml). During each distention period the behavioural observations are presented. bas = baseline and post = post distention period. Data are presented as means ± SEM.

Figure 3. Threshold volumes of distention (ml) to induce discomfort behaviour, pain behaviour, increase and decrease in baseline EMG signal. Data are presented as means ± SEM.

Figure 4. Individual tracing of a raw, filtered and rectified EMG waveform before, during and after staircase distention (0.1 to 0.6 ml).

Figure 5. Changes in maximal amplitude of EMG (MAX) and area under curve (AUC) as percentage to baseline (= 100%) induced by staircase increases in distention volume (ml). Data are presented as means ± SEM.

Figure 6. Changes in mean arterial pressure (MAP in mm Hg) and heart rate (HR in beats/min) induced by staircase increases in distention volume (ml). During each distention period the behavioural observations are presented. bas = baseline and post = post distention period. Data are presented as means ± SEM.

Figure 7. Correlation between mean arterial pressure (MAP in mm Hg) and MAX (mV) or AUC (mV x sec) during the staircase distention model.

Figure 8. Changes in gross activity (counts/min) induced by phasic increases in distention volume (ml). During each distention period the behavioural observations are presented. bas = baseline and int = un-inflated interval. Data are presented as means ± SEM.

Figure 9. Individual tracing of a raw, filtered and rectified EMG waveform before, during and after phasic distention (0.1, 0.3 and 0.5 ml).

Figure 10. Changes in maximal amplitude of EMG (MAX) and area under curve (AUC) as percentage to baseline (= 100%) induced by phasic increases in distention volume (ml). Data are presented as means ± SEM.

Figure 11. Changes in mean arterial pressure (MAP in mm Hg) and heart rate (HR in beats/min) induced by phasic increases in distention volume (ml). During each distention period the behavioural observations are presented. bas = baseline and int = un-inflated interval. Data are presented as means ± SEM.

Figure 12. Correlation between mean arterial pressure (MAP in mm Hg) and MAX (mV) or AUC (mV x sec) during the phasic distention model.

Figure 13. Changes in maximal amplitude of EMG (MAX) and area under curve (AUC) as percentage to baseline (= 100%) induced by phasic increases in distention volume (ml) after pre-treatment with morphine (0.3, 1.5 and 3 mg/kg). Data are presented as means ± SEM.

Figure 14. Step-down latency (sec) of distended (0.6 ml) and non-distended rats exposed to a small platform in an open field for 9 trails. Data are presented as means ± SEM.

DETAILED DESCRIPTION

This invention relates to an animal model to measure visceral pain by means of a balloon catheter and an implantable sensor module having transcutaneous telemetring ability. The implantable sensor is capable to transmit data relevant to visceral pain to an apparatus outside the body capable of continuously monitoring the user's status and is capable to accept a plurality of input signals either simultaneously or sequentially, preferably from bipolar electrode pairs and blood catheters. As such, this model provides an adequate tool to measure visceromotor and pseudoaffective responses to visceral pain continuously and simultaneously in a non-human animal. In particular it relates to a non-human animal model to measure visceral pain comprising a balloon catheter and an implantable sensor module having transcutaneous telemetring ability. Preferably, a non-human animal wherein both the balloon catheter and sensor module are chronically implanted in said animal. Preferably, the balloon catheter is implanted in the duodenum and the sensor module is connected to a bipolar electrode pair and a blood catheter.

The balloon catheter used in the present model is an implantable balloon catheter characterized in that the balloon catheter consists of biocompatible material such as non-immunogenic polymeric material of poly-para-xylylene, polyethylene, natural or synthetic rubber, silicone or other aromatic based moiety having a membrane portion

with a porosity effective to block passage of immunogenic agents. The balloon catheter of the present invention consists of biocompatible tubing (1) closed at one end with elastic material (2). To improve the introduction of the balloon catheter into the animal according to the method of the present invention, the elastic material is attached to the biocompatible tubing at a position (3) proximal from the tube end (4). In a further improvement to enhance the radial expansion of the elastic material, the elastic material is also attached at the end of the biocompatible tubing (5) and said tubing end is rigidly sealed (6). To allow balloon inflation in this last configuration, the tubing end distal from attachment point (3) is foreseen from a number of holes (7). A further characteristic of the balloon catheter according to the invention is that it comprises fixation means to prevent movement of the catheter due to the peristaltic movement of the intestinal tract. Said fixation means are positioned proximal from the tube end to allow fixation of the tubing to the stomach wall of the animal. In a preferred embodiment the fixation means consist of two nodes (8).

The diameter and length of the tubing are determined by the test animal used. As in the method according to the invention the implantable balloon catheter is introduced in the duodenum of the test animal, the length of the tubing should permit to reach from the duodenum of said animal, via the stomach wall, to the skull where it is accessibly immobilized. For example in rat the length of the tubing would be between 15 and 50 cm, preferably between 20 and 30 cm with an outside diameter from 2 – 2.5 mm.

The implantable sensor modules with transcutaneous telemetring ability that can be used in the animal model according to the invention are known in the art. For example, there are pacemakers available which, when implanted and connected to the heart, can monitor electrocardial activity through electrodes attached to the pacemakers. The electrodes function as electropotential sensors, and the pacemaker include interface circuits which buffer the sensor signals, formats them and transmits then the formatted signals by way of a bi-directional radiofrequence (RF) communication link to an external communication module. The telemetered signals are monitored and processed through the external module.

Further it is known in the art to provide for enablement of two or more functions with implanted telemetric devices allowing the sequential or simultaneous measurement

of up to eight individual parameters. In the animal model according to the present invention the implantable sensor module is capable of accepting a plurality of input signals either sequentially or simultaneously. In a preferred embodiment the sensor module comprises at least two input ports and is connected to at least one bipolar electrode pair and at least one blood catheter. In a more preferred embodiment the sensor module is a radiotelemetric device connected to a bipolar electrode pair for electromyography measurement of the abdominal muscle and connected to a blood catheter set up to register mean arterial pressure and heart rate of the abdominal aorta through the femoral artery. It will be readibly appreciated by the skilled person that the implantable sensor module as used herein requires an external module to detect and demodulate the output signal of the sensor module into an output signal suitable for driving an output graphics device. For example a recorder for recording the variations in amplitude of a current over time.

It is thus an object of the present invention to provide a system for measuring visceral pain comprising; a balloon catheter, an implantable sensor having transcutaneous telemetring ability, and an external module capable to monitor and process the telemetered signals. A system wherein the balloon catheter is implanted in the duodenum of a test animal and wherein the implantable sensor module is set up to receive both visceromotor (EMG) and pseudoaffective (MAP, HR) responses of the test animal. In a particular embodiment the system comprises a radiotelemetric device connected to a bipolar electrode pair for electromyography measurement of the abdominal muscle and connected to a blood catheter set up to register mean arterial pressure and heart rate of the abdominal aorta through the femoral artery.

In a further embodiment this invention provides kits comprising a balloon catheter, an implantable telemetric sensor module, a bipolar electrode pair and a blood catheter, for use in a method to produce an animal model to measure visceral pain.

It is thus a further object of the present invention to provide a method for producing an animal model to measure visceral pain comprising; implanting a balloon catheter in the duodenum of said animal; and implanting a telemetric sensor

module in the abdominal cavity of said animal wherein said telemetric sensor module is set up to receive input signals form at least one bipolar electrode pair and at least one blood catheter. A process for producing an animal model according to the invention wherein the balloon catheter is fixated to the stomach wall of the test animal using the fixation means (8) of the implantable balloon catheter. A process for producing an animal model according to the invention wherein the biocompatible tubing (1) of the implantable balloon catheter is guided to the skull of the test animal, preferably subcutaneously, where it is accessibly immobilized. In a preferred embodiment the tubing end is fixed to a syringe connecter, preferably a connecter with a 90 ° loop which are commercially available. The syringe connecter is fixated to the skull of the test animal, for example using dental cement. A process for producing an animal model according to the invention wherein the electrodes of the bipolar electrode pair is sutured into the abdominal muscle for EMG measurements. A process for producing an animal model according to the invention wherein the blood catheter is tunneled into the abdominal aorta, preferably through the femoral artery.

This invention will be better understood by reference to the Experimental Details that follow, but those skilled in the art will readily appreciate that these are only illustrative of the invention as described more fully in the claims that follow thereafter. Additionally, throughout this application, various publications are cited. The disclosure of these publications is hereby incorporated by reference into this application to describe more fully the state of the art to which this invention pertains.

EXPERIMENTAL

MATERIAL AND METHODS

Animals

Naive male albino Wistar rats (WU; Harlan, The Netherlands) weighing 280-300 g at the beginning of the experiments, were used. Rats were housed individually in a Macrolon individual ventilated cage (25 x 40 x 22 cm) containing a layer of wood shavings under conditions of constant ambient temperature (21 ± 1°C), constant humidity (60 ± 15%) and light/dark rhythm (with lights on from 7 a.m. to 7 p.m.). After surgery, the animals were housed individually under presurgical conditions. Food (complete laboratory chow) and water were accessible *ad libitum* throughout the experiment.

Surgery

Rats were equipped with a balloon catheter in the duodenum to induce duodenal distention and a telemetric transmitter to study abdominal electromyography (EMGs), mean arterial pressure (MAP), heart rate (HR) and body activity. Operations were performed under fentanyl/fluanisone anesthesia (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium; 0.1 ml/100 g body weight (BW), intramuscularly) and Midazolam hydrochloride (Dormicum®, Hoffman-LaRoche, Mijdrecht, The Netherlands; 0.05 ml, intraperitoneally (ip)) as a muscle relaxant. Before the muscle relaxant was injected, the analgesic effect of fentanyl anesthesia was tested in the rat by checking for the absence of its pedal and cornea reflexes. Total absence of the pain response normally appeared after 10 min and then the muscle relaxant was injected. Surgery was performed in a sterile laminar flow cabinet to minimize risk of infection. A small longitudinal incision was made on the linea alba at the anterior of the abdomen. A self-made silicone balloon catheter (i.d. 1.02 mm/ o.d. 2 mm) was chronically implanted into the duodenum according to the procedure described by Colburn et al. (1989), with some modifications (see figure 1). A small incision (3 mm) was made in the stomach wall for insertion of the catheter, which in turn was guided into the duodenum until the small node on the

balloon catheter has just crossed the stomach wall. Then the stomach wall was sutured tightly between the two nodes to fixate the catheter. The other end of the balloon catheter was tunneled subcutaneously to the skull, where it was fixed to a connector (Bilaney, Dusseldorf, Germany) with a 90 ° loop. There it was fixated to the skull with dental cement (Dentimex BV, Zeist, The etherlands). A telemetry transmitter (TL11M2-C50-PXT, Data Sciences International, St. Paul, USA), consisting of a bipolar electrode pair and blood catheter, was chronically implanted into the abdominal cavity of the rat. The non-insulated tips (helix of stainless steel wire; Ø 0.45 mm, 8 mm) of the electrodes were sutured in parallel (5 mm inter-electrode space) into the abdominal muscle for EMG measurements, whereas the blood catheter was tunneled through the femoral artery to the abdominal aorta to register MAP and HR. Postoperatively the animals received 0.1 mg/kg of the long-acting opiate analgesic Buprenorfine hydrochloride (Temgesic®, Reckitt & Colman, Kingston-upon-Hull, UK; 0.1 ml, subcutaneously).

Experimental design

Rats (n=14) were surgically equipped with a duodenal balloon catheter and telemetric transmitter and were allowed to recover from surgery for 12 days. During the recovery period the animals were handled every day for weighing and habituation purposes. Rats were accustomed to experimental procedures (twice before the experiment). During the experiment, EMG, body activity, MAP and HR were simultaneously and continuously recorded before (baseline), during and after (post) duodenal distention. Baseline values were recorded for 30 sec. Subsequently, rats received a volume-fixed duodenal distention protocol of 300 (staircase n=7) or 690 (phasic n=7) sec (see below). Afterwards, post recordings were performed for another 120 (staircase) or 300 (phasic) sec.

To validate our model, an additional experiment was performed with a new group of rats. Thirty min prior to phasic duodenal distention, rats were ip injected with morphine (0.3 (n=8), 1.5 (n=7) or 3 (n=8) mg/kg body weight) or saline (n=8).

In a final experiment to validate our model, hereinafter referred to as the passive avoidance test, 12 rats were surgically equipped with a duodenal balloon catheter and were allowed to recover from surgery for 12 days. These animals were handled every day for weighing and habituation purposes. In the passive avoidance test, the step down latency was scored.

All experiments were performed in the home cage during the light phase of the circadian cycle between 8 a.m. and 12 p.m. After the experiment, all rats were killed by an overdose (0.5 ml) of pentobarbital (Nembutal®, Sanofi, Belgium; 60 mg/ml, ip), dissected and macroscopically inspected for infections. In none of the animals any signs of infection were found.

Duodenal distention

Forty-five min prior to baseline recordings, the skull connector of the balloon catheter was attached to a fluid-filled long-line (1 m; polyethylene tubing, Becton Dickinson, UK) with a syringe, so that variable volume-fixed distentions could be delivered from outside the home cage without restraining the rat. During this habituation period, rats remained to sleep, so that stress-free baseline recordings were started. Two protocols were used: 1) staircase distention: the balloon was inflated with increasing volumes of 0.1 ml (each 30 sec), starting from 0.1 to 0.6 ml; 2) phasic distention: inflation of 0.1, 0.3 and 0.5 ml for 30 sec with resting un-inflated intervals of 5 min.

Behavioral measurements

During the experiment, the behavioral response to duodenal distention in the home cage was scored by the experimentator. Following behavioral scores were used: sleep, wake up, alert, shake, explore, groom abdominal region, abdominal contractions, stretching behavior (stretches of the torso and hyperextension of the hind limbs with concave arching of the back) and immobility. In the staircase model, the threshold (distention volume (ml)) inducing discomfort (shake, explore) and/or pain (groom abdominal region, stretching and immobility) behavior was determined.

Passive avoidance test

To validate whether duodenal distention is perceived as aversive, rats (n=12) were exposed to a passive avoidance test. On day 1, the rat was palced on a small platform, which was located in a large open field (Ø 80 cm). When the rat stepped down the platform, it was removed from the open field. For habituation purposes, this procedure was repeated for each rat in 9 learning trails with an interval of 10 min. When the rat did not step down the platform, it was removed after 150 sec. The next day, the same rats were submitted to the same procedure (no distention, control group; n=6) or received a duodenal distention of 0.6 ml for 5 sec immediately after stepping down the platform (n=6). This procedure was repeated in each rat for 9 learning trails with an interval of 10 min. An increase in step down latency can be used as an indication for increased pain perception.

Telemetric measurements

Body activity was telemetrically measured by detecting changes in signal strength of the transmitter that occurred as the animal moved about its cage. EMGs, body activity, MAP and HR were registered by the data acquisition program ART 2.2 (Data Sciences International, St. Paul, USA). Raw EMG activity was continuously collected as a waveform (at a frequency of 1000 Hz), was low cut filtered at 50 Hz to eliminate movement interference and fully rectified by Spike2, version 4.11 (Cambridge Electronic Design, Cambridge, UK). From the rectified EMG, area under the curve (AUC; mV x sec) and maximal value (EMG_{max}; mV) were analyzed. In the staircase model, the threshold (distention volume (ml)) inducing an increase or decrease in baseline EMG amplitude was determined.

Statistics

Distention thresholds (ml), EMG data (% to baseline), gross activity (counts/min), MAP (mmHg) and HR (beats/min) are presented as means ± SEM. Cardiovascular data of each single rat are averaged to 1 point per baseline and distention period, before statistical analysis is performed by one-way Analysis of Variance (ANOVA) and post-hoc Student's t-test. For the morphine experiment, a two-factor multiple ANOVA (MANOVA) with repeated measured was used. P values of < 0.05 were considered significant.

In the phasic distention protocol, one rat was excluded for statistical analysis on cardiovascular data due to bad signaling.

RESULTS AND DISCUSSION

Staircase model

Behavioral and Visceromotor response

Staircase distention of the duodenum produced "discomfort" behavior (shaking, exploring) starting at a volume of 0.2 ml. At higher volumes (0.4 to 0.6 ml) rats showed "pain" behavior (grooming abdominal region, stretching, immobility) additionally (figure 2 and 3). Abdominal contractions are shown before the occurrence of pain-related behavior. Figure 4 shows an example of an individual tracing of the raw, filtered an rectified EMG signal before (basal), during (0.1 to 0.6 ml) and after (post) staircase distention. "Active" behavior (shaking, exploring, grooming, abdominal cramps) was accompanied by a significant increase in EMG amplitude, whereas stretching behavior during higher distention volumes (0.4 to 0.6 ml) was reflected in a decrease of the baseline EMG signal (figure 3 and 4).

Staircase distention produced a volume-dependent increase in EMG_{max} and AUC (figure 5). One-way ANOVA on EMG_{max} ($F(6, 48)=2.9$, $p < 0.05$) and AUC ($F(6, 48)=3.1$, $p < 0.05$) showed significance and post-hoc analysis revealed a significant increase in AUC and EMG_{max} as compared to baseline at 0.2 ($p<0.05$), 0.3 ($p<0.05$), 0.4 ($p<0.05$), 0.5 ($p < 0.005$) and 0.6 ($p < 0.005$) ml distention volume.

Cardiovascular response

Staircase distention of the duodenum produced a volume-dependent increase in MAP (figure 6A). One-way ANOVA on MAP showed significance ($F(6, 48)=22.6$, $p <0.0001$) and post-hoc analysis revealed a significant increase in MAP as compared to baseline at 0.3 ($p < 0.005$), 0.4 ($p < 0.0001$), 0.5 ($p < 0.0001$) and 0.6 ($p < 0.0001$) ml distention volume.

Staircase distention of the duodenum produced a volume-dependent increase in HR (figure 6B). One-way ANOVA on HR showed significance ($F(6, 48)=3.9$, $p < 0.005$) and post-hoc analysis revealed a significant increase in HR as compared to baseline at 0.3 ($p < 0.05$), 0.4 ($p < 0.01$), 0.5 ($p < 0.005$) and 0.6 ($p < 0.01$) ml distention volume.

Relationship visceromotor – pseudoaffective response

Figure 7 shows the positive correlation between mean MAP and EMG_{max} (MAP=63 EMG_{max} + 109, r = 0.9) or AUC (MAP=143 AUC + 87, r = 0.8) during staircase distention. Mean MAP also showed a positive correlation with HR (HR=1.2 MAP + 242, r = 0.9) and mean HR showed a positive correlation with EMG_{max} (HR=75 EMG_{max} + 375, r = 0.8) and AUC (HR=185 AUC + 343, r = 0.8); data not shown.

Phasic model

Behavioral and Visceromotor response

Phasic distention of the duodenum produced "discomfort" and "pain" behavior at a volume of 0.3 and 0.5 ml (figure 8). Stretching behavior was less reflected in a reduction of the basal EMG amplitude (see figure 9) as shown in the staircase protocol. Phasic distention produced a volume-dependent increase in EMG_{max} and AUC (figure 10). One-way ANOVA on EMG_{max} ($F(3, 27)=4.4$, $p < 0.05$) and AUC ($F(3, 27)=3.8$, $p < 0.05$) showed significance and post-hoc analysis revealed a significant increase in AUC and EMG_{max} as compared to baseline at 0.3 ($p<0.05$) and 0.5 ($p < 0.01$) ml distention volume.

Cardiovascular response

Phasic distention of the duodenum produced a volume-dependent increase in MAP (figure 11A). One-way ANOVA on MAP showed significance ($F(3, 23)=11.8$, $p <0.0001$) and post-hoc analysis revealed a significant increase in MAP as compared to baseline at 0.5 ($p < 0.005$) ml distention volume.

Phasic distention produced a volume-dependent increase in HR (figure 11B). One-way ANOVA on HR showed significance ($F(3, 23)=5.8$, $p < 0.005$) and post-hoc analysis revealed a significant increase in HR as compared to baseline at 0.3 ($p<0.005$) and 0.5 ($p < 0.05$) ml distention volume.

Relationship visceromotor – pseudoaffective response

Figure 12 shows the positive correlation between mean MAP and EMG_{max} ($MAP=26\ EMG_{max} + 101$, $r = 0.8$) or AUC ($MAP=64\ AUC + 90$, $r = 0.8$) during phasic distention. Mean MAP also showed a positive correlation with HR ($HR=1.5\ MAP + 204$, $r = 0.7$) and mean HR showed a positive correlation with EMG_{max} ($HR=67\ EMG_{max} + 347$, $r = 0.98$) and AUC ($HR=152\ AUC + 321$, $r = 0.95$); data not shown.

Morphine experiment

Pre-treatment with morphine inhibited distention-induced pain behavior (stretching behavior, grooming abdominal region, immobility; $p<0.0001$, data not shown).

Morphine treatment dose-dependently reduced the distention-induced increase in EMG_{max} and inhibited the distention-induced increase in AUC (figure 13). MANOVA revealed a significant treatment effect on EMG_{max} ($F(3,27)=4.8$, $p < 0.01$) and AUC ($F(3,27)=4.3$, $p < 0.05$), significant distention effect on EMG_{max} $F(2,26)=14.9$, $p < 0.0001$) and AUC ($F(2,26)=10.8$, $p < 0.0005$) and significant distention x treatment interaction on EMG_{max} ($F(6,52)=3.0$, $p < 0.05$) and AUC ($F(6,52)=2.8$, $p < 0.05$). Post-hoc MANOVA showed a significant decrease of the EMG_{max} by morphine treatment at doses of 0.3, 1.5 and 3 mg/kg ($p<0.05$). Post-hoc MANOVA showed a significant decrease of the AUC by morphine treatment at doses of 1.5 and 3 mg/kg ($p<0.05$) and a tendency in AUC reduction by 0.3 mg/kg morphine treatment ($p = 0.07$).

Passive avoidance test

To evaluate the afferent pathway conveying acute duodenal nociception and cortical pain perception, one can stucy the animal behavioral responses to pain. In this study we employed a passive avoidance test to evaluate noxious related behaviour. In agreement with previous studies, our study shows that duodenal distension increases the step-

down latency (Figure 14 shows the step-down latency of rats in the passive avoidance test on day 2. Duodenal distention with a volume of 0.6 ml resulted in a significant increase in the step-down latency as compared to non-distended rats ($F(1,10)=7.3$, $p<0.05$)). Taken together with the above mentioned behavioral data, the present study indicates that duodenal distention in our animal model is perceived as a noxious, aversive stimulus.

Conclusion

The present data show that radiotelemetry is an adequate tool to measure visceromotor and cardiovascular responses to visceral pain continuously and simultaneously in the conscious and freely moving rat, without additional handling-related or restraint stress. Duodenal distention induced "discomfort" and "pain" behavior and a volume-dependent visceromotor and cardiovascular response in the rat.

The staircase distention model is suitable for studying the threshold of aversive behavioral (grooming abdominal region, stretching), visceromotor (abdominal contractions, increase in EMG amplitude and AUC) and pseudoaffective (MAP and HR) responses to duodenal distention. The cardiovascular and visceromotor responses to duodenal distention are both useful measures of visceral nociception and abolition of both responses by a drug is predictive of antinociceptive efficacy. Both the staircase and phasic distention models are suitable for studying the potency of new pharmacological compounds on reversing visceral nociception.

WHAT IS CLAIMED IS:

1. An animal model for measuring visceral pain comprising a balloon catheter and an implantable sensor module having transcutaneous telemetring ability.

2. An animal model according to claim 1 wherein the balloon catheter is an implantable balloon catheter.

3. An animal according to claim 2 wherein the implantable balloon cathether comprises fixation means preferably consisting of two nodes to fixate the catheter.

4. An animal according to claim 2 wherein the balloon catheter is implanted into the duodenum.

5. An animal according to any of the preceding claims, wherein the implantable sensor module is capable of accepting a plurality of input signals.

6. An animal according to claim 5 wherein the implantable sensor module is set up to receive both visceromotor and pseudoaffective responses of the test animal.

7. An animal according to claim 5 wherein the implantable sensor comprises at least two input ports.

8. An animal according to claim 5 wherein the implantable sensor is connected to a bipolar electrode pair and a blood catheter.

9. A system for measuring visceral pain comprising:

a balloon catheter;

an implantable sensor module having transcutaneous telemetring ability; and

an external module capable to monitor and process the telemetered signals.

10. A system according to claim 9 wherein the balloon catheter is implanted in the duodenum of the test animal; and wherein the implantable sensor module is set up to receive both visceromotor and pseudoaffective responses of the test animal.

11. A kit for generating an animal according to claim 1 comprising a balloon catheter; an implantable sensor module having transcutaneous telemetring ability; a bipolar electrode pair; and a blood catheter.

ABSTRACT

ANIMAL MODEL TO EVALUATE VISCERAL PAIN PERCEPTION

This invention relates to an animal model to measure visceral pain by means of a balloon catheter and an implantable sensor module having transcutaneous telemetring ability. The implantable sensor module according to the invention is set up to receive both visceromotor and pseudoaffective responses of the test animal. In particular, this invention provides a non-human animal model wherein balloon catheter is an implantable balloon catheter, preferably implanted in the duodenum of the test animal and the implantable sensor is set up to receive input signals form at least one bipolar electrode pair and at least one blood catheter.

It is thus a further object of the present invention to provide a method for producing said animal as well as kits comprising a balloon catheter and an implantable sensor module for use in a method for producing said animals.

Fig. 1

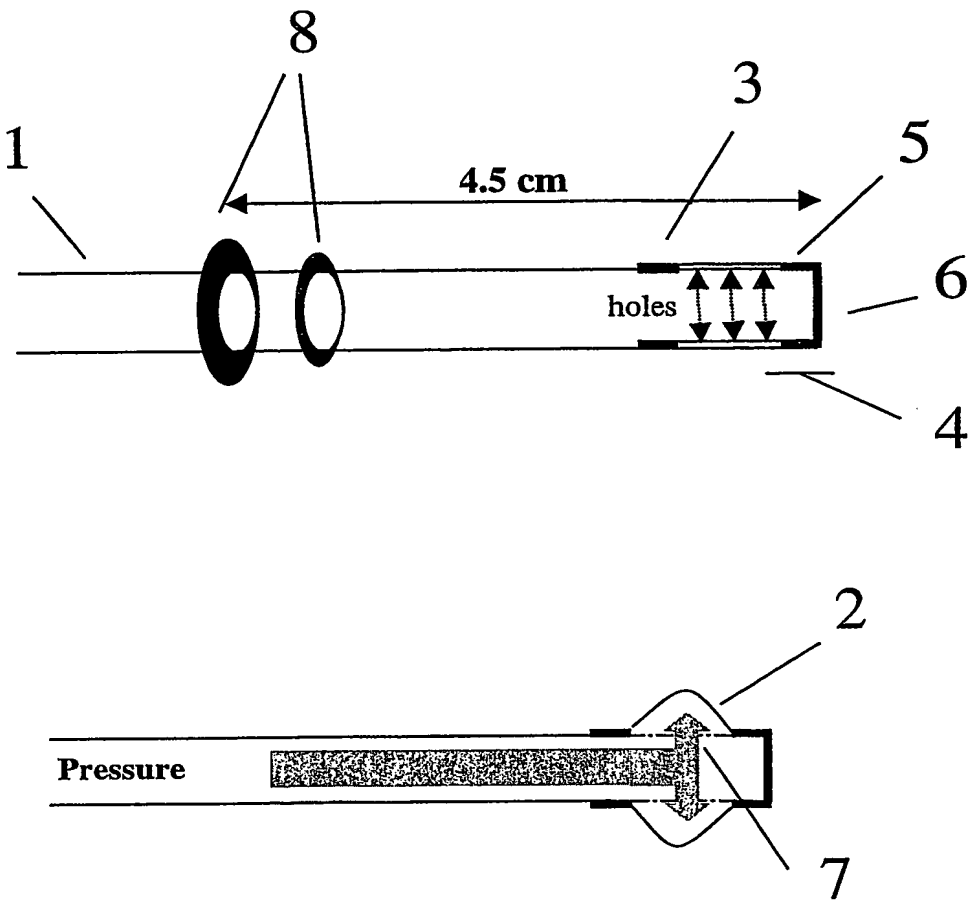


Fig. 2

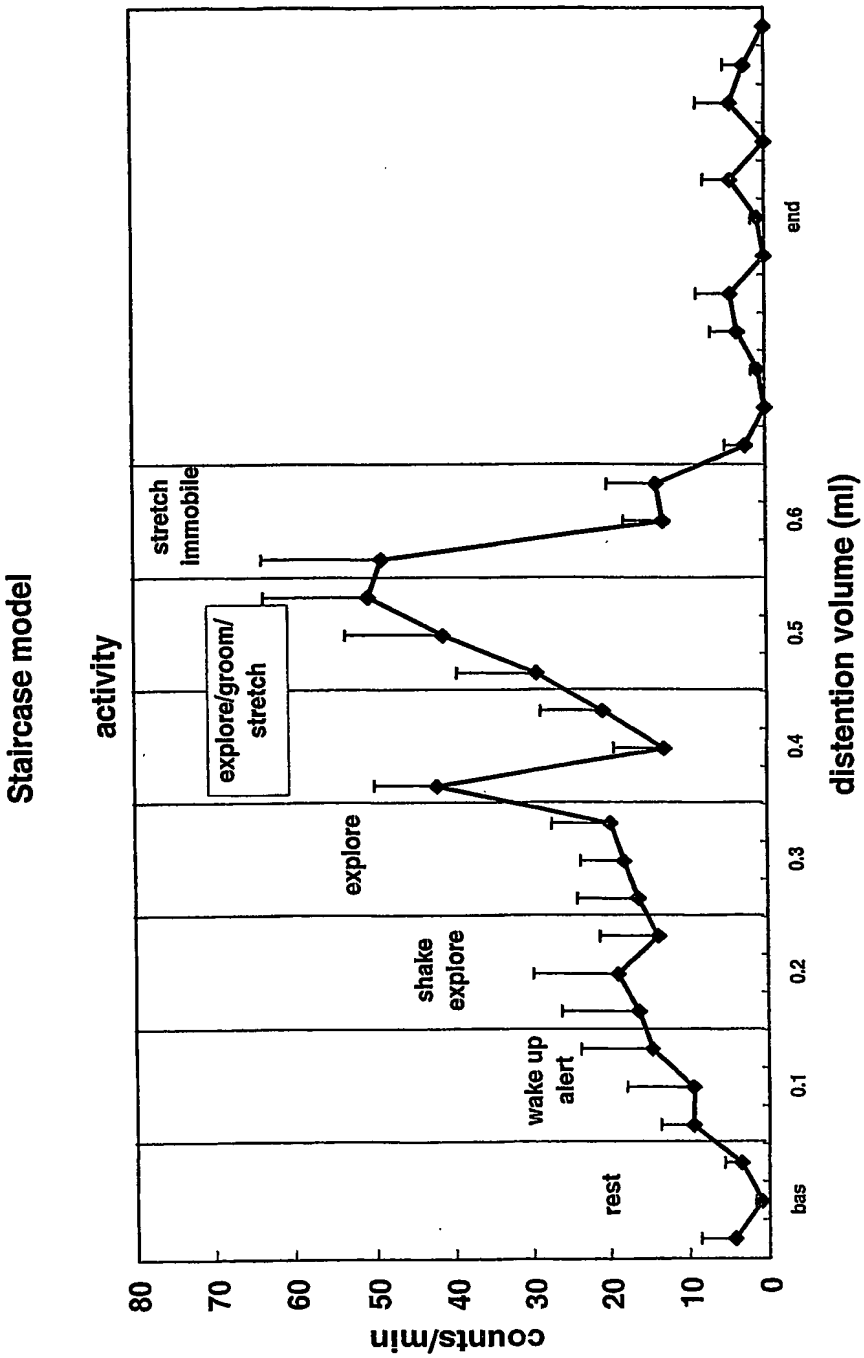

Staircase model
activity
counts/min
distention volume (ml)
bas
0.1
0.2
0.3
0.4
0.5
0.6
end
rest
wake up alert
shake explore
explore
explore/groom/ stretch
stretch immobile
0
10
20
30
40
50
60
70
80

Fig. 3

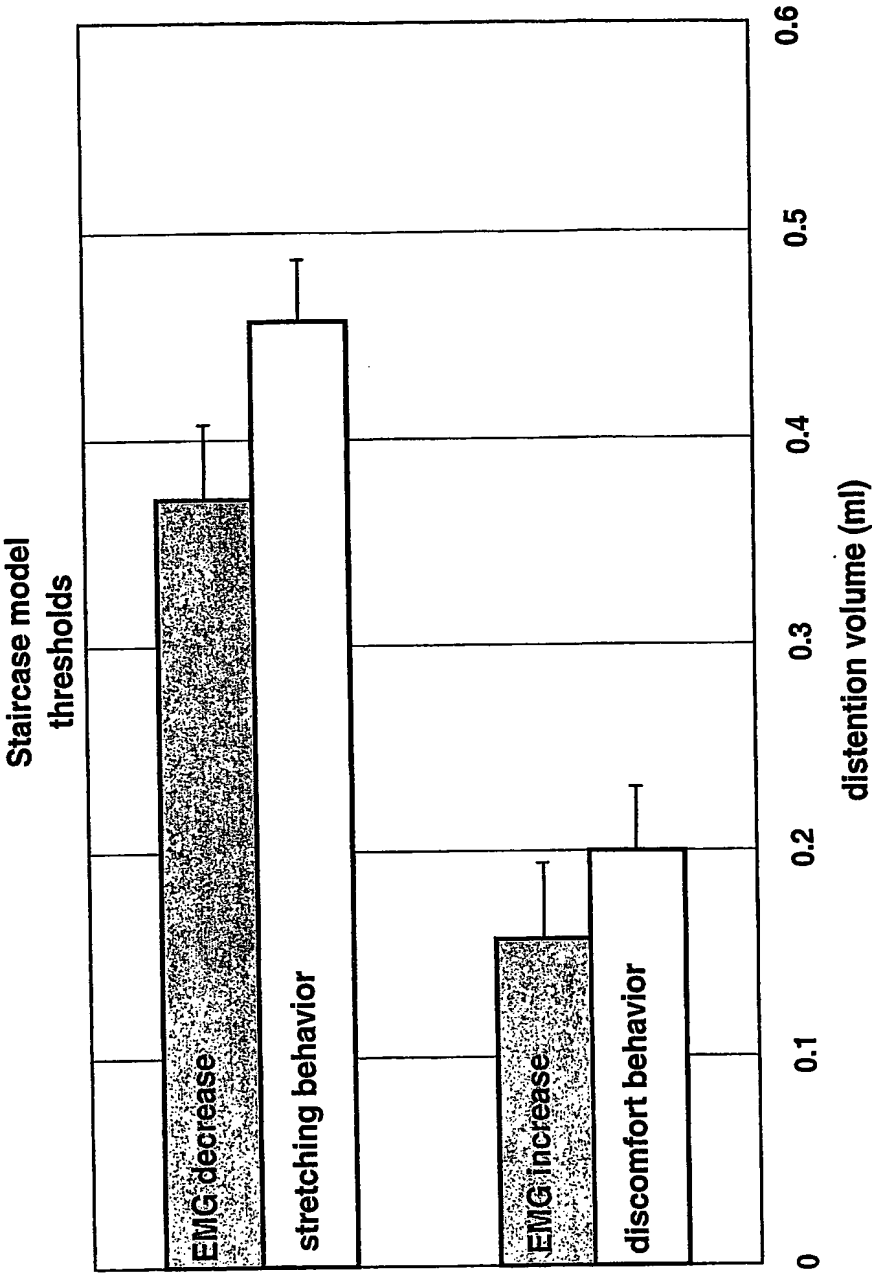

Staircase model thresholds
EMG decrease
stretching behavior
EMG increase
discomfort behavior
0
0.1
0.2
0.3
0.4
0.5
0.6
distention volume (ml)

Fig. 4

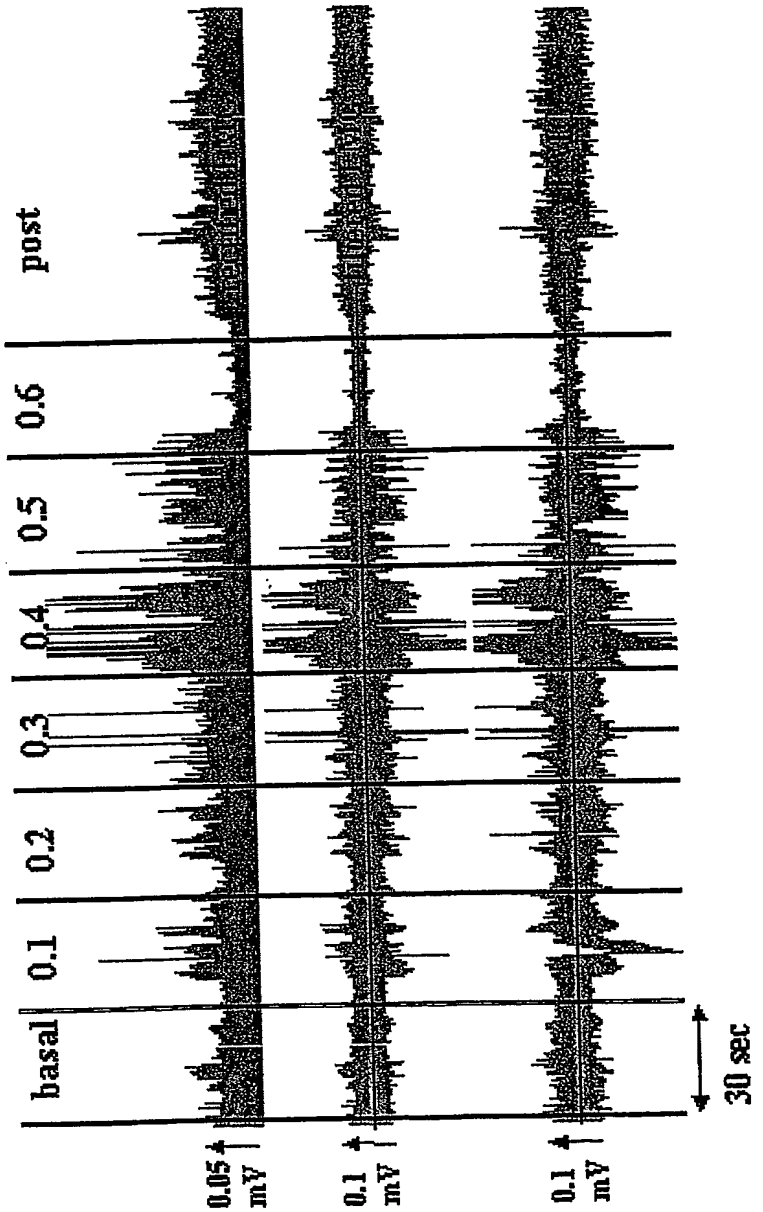


Fig. 5A

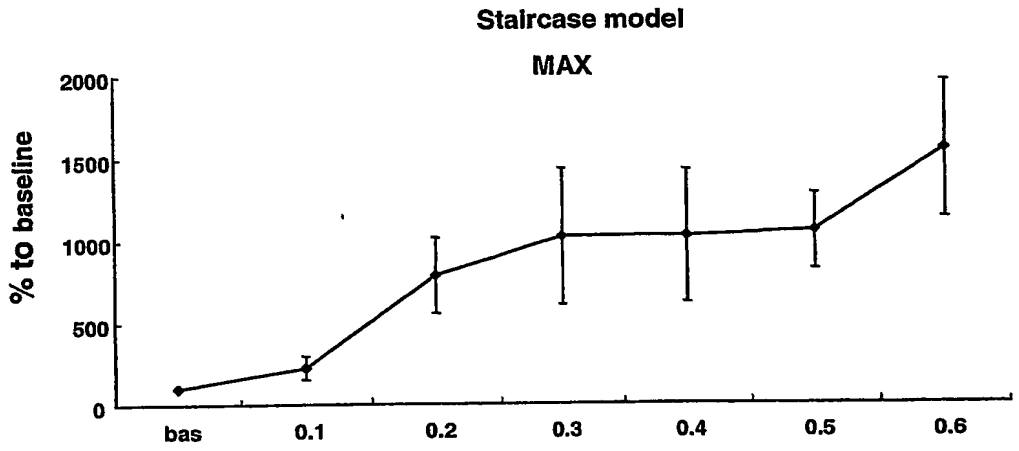

Staircase model
MAX
% to baseline
2000
1500
1000
500
0
bas
0.1
0.2
0.3
0.4
0.5
0.6

Fig. 5B

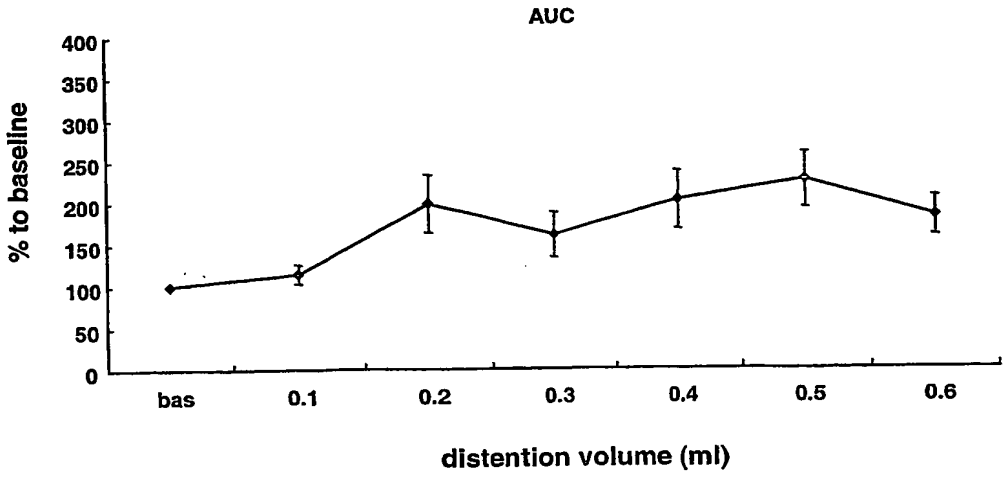

AUC
% to baseline
400
350
300
250
200
150
100
50
0
bas
0.1
0.2
0.3
0.4
0.5
0.6
distention volume (ml)

Fig. 6A

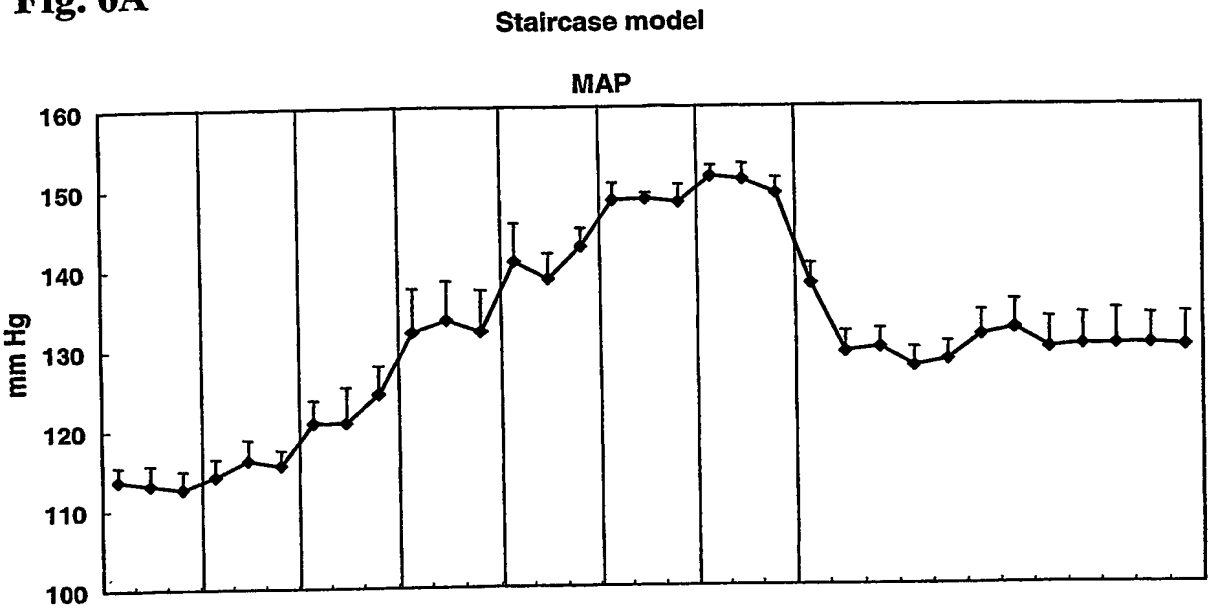

Staircase model
MAP
160
150
140
130
120
110
100
mm Hg

Fig. 6B

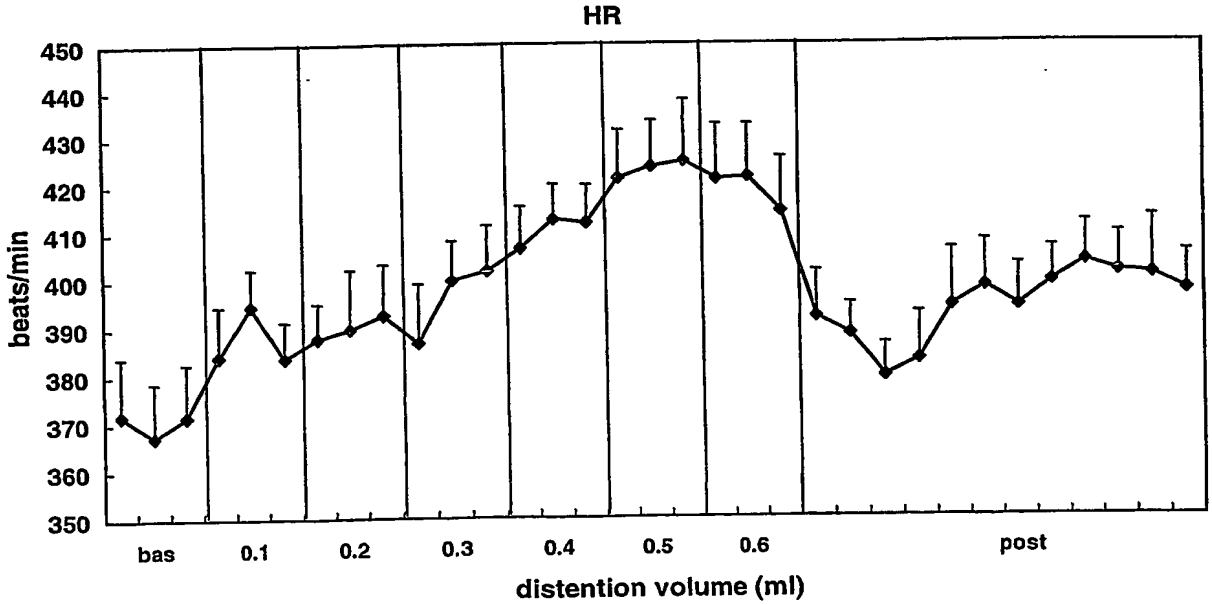

HR
450
440
430
420
410
400
390
380
370
360
350
beats/min
bas
0.1
0.2
0.3
0.4
0.5
0.6
post
distention volume (ml)

Fig. 7A

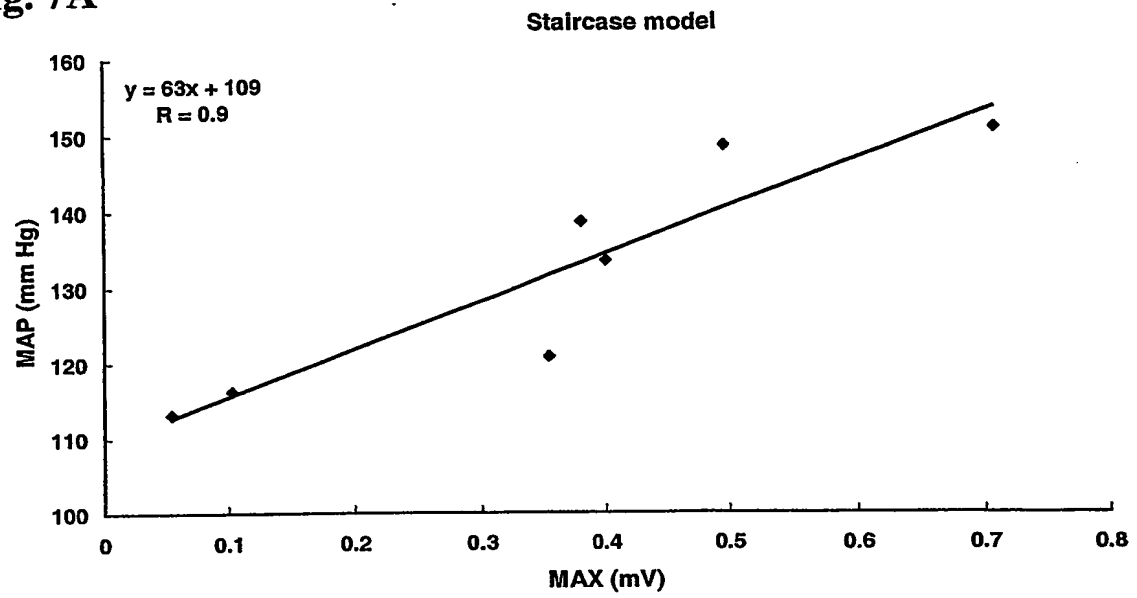

Staircase model
y = 63x + 109
R = 0.9
MAP (mm Hg)
MAX (mV)
160
150
140
130
120
110
100
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

Fig. 7B

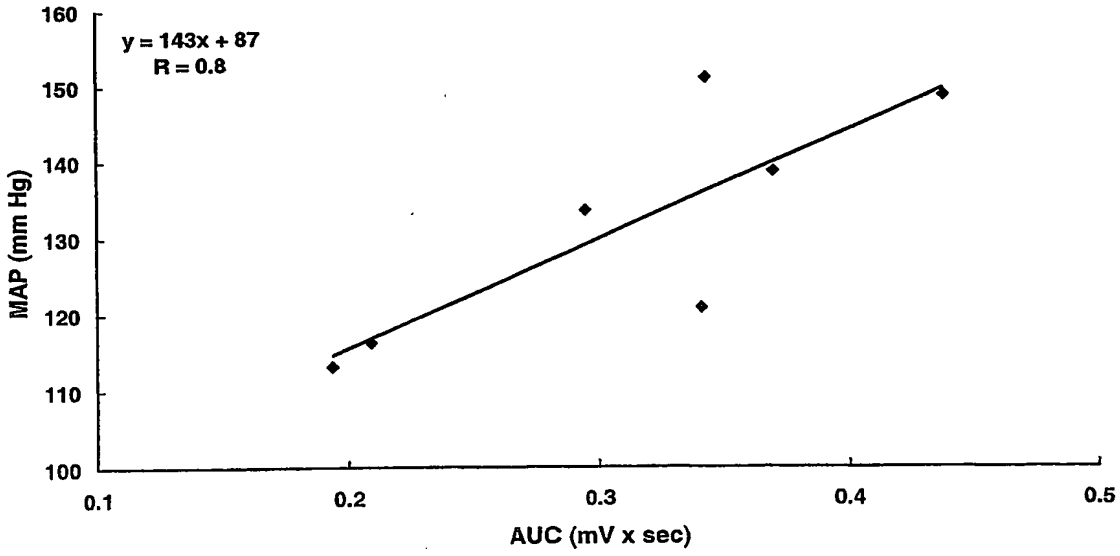

y = 143x + 87
R = 0.8
MAP (mm Hg)
AUC (mV x sec)
160
150
140
130
120
110
100
0.1
0.2
0.3
0.4
0.5

Fig. 8

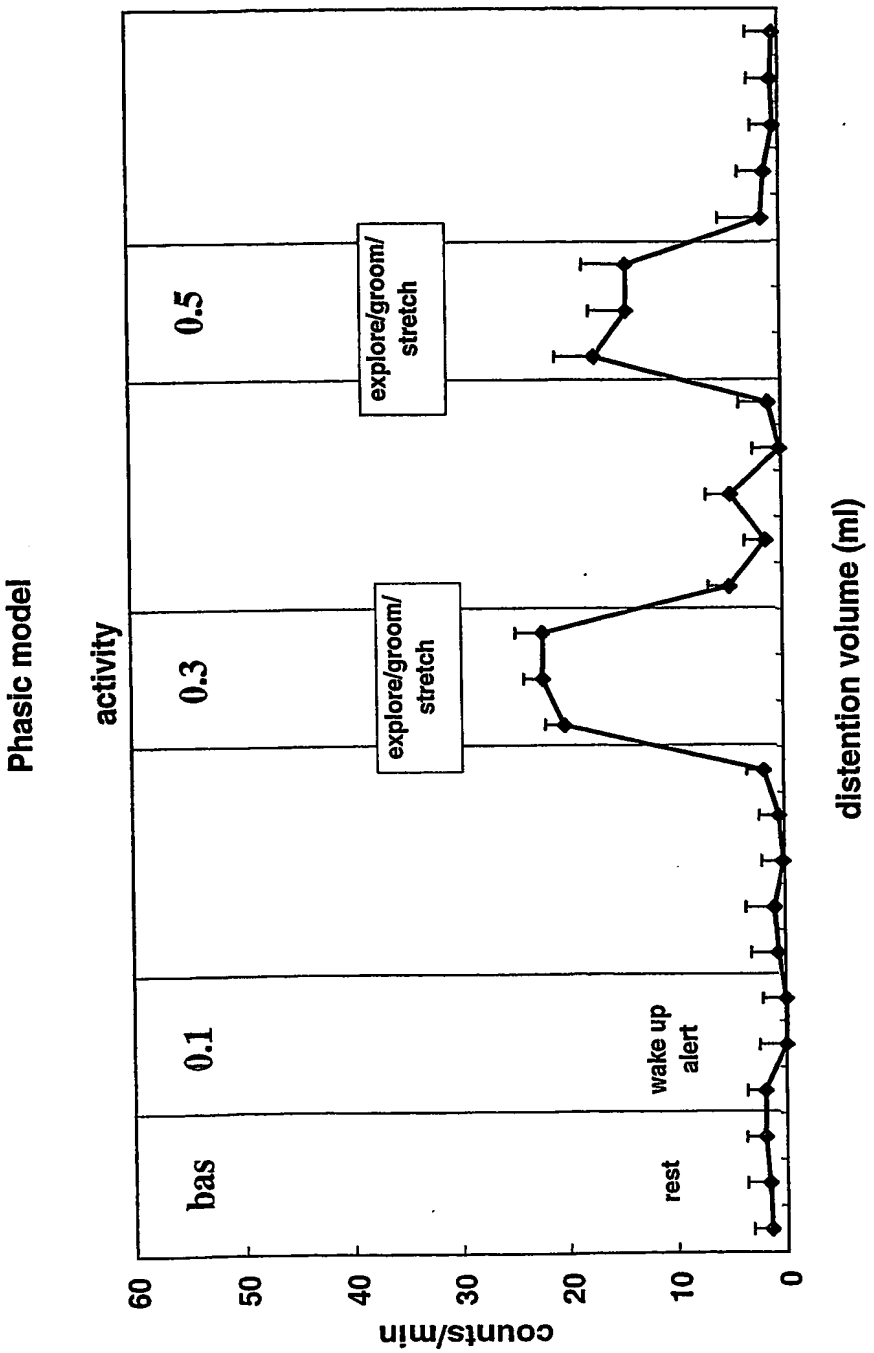

Phasic model
activity
bas
0.1
0.3
0.5
rest
wake up alert
explore/groom/ stretch
explore/groom/ stretch
counts/min
60
50
40
30
20
10
0
distention volume (ml)

Fig. 9

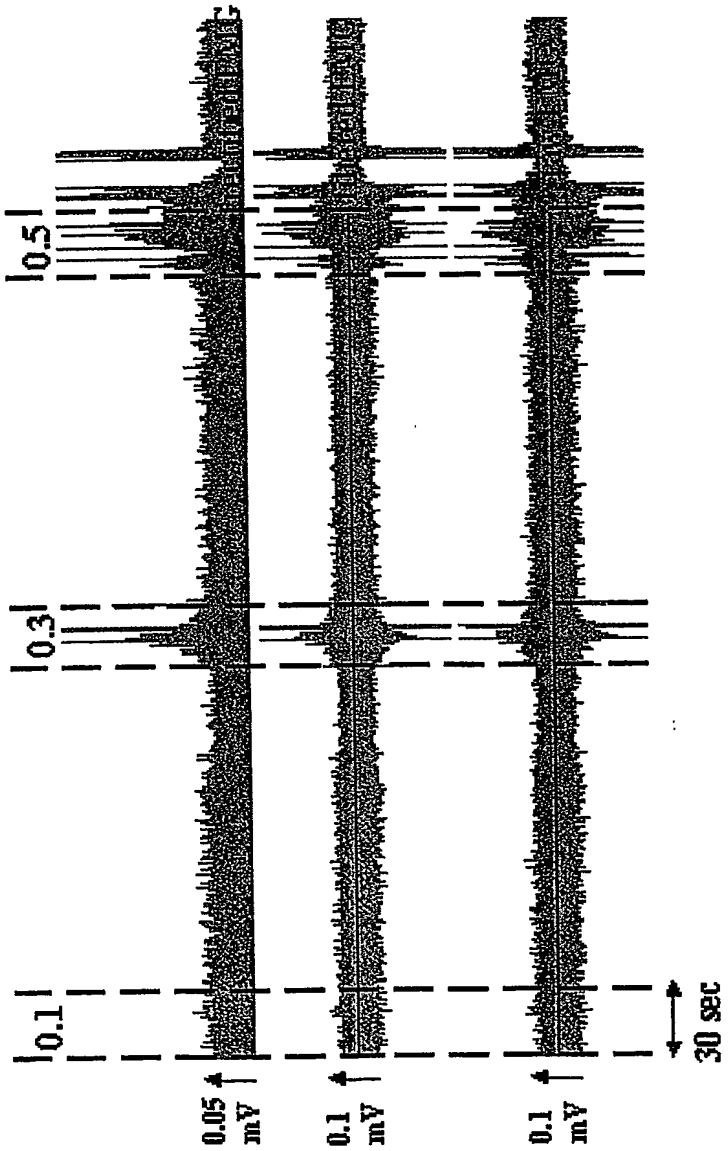


Fig. 10A

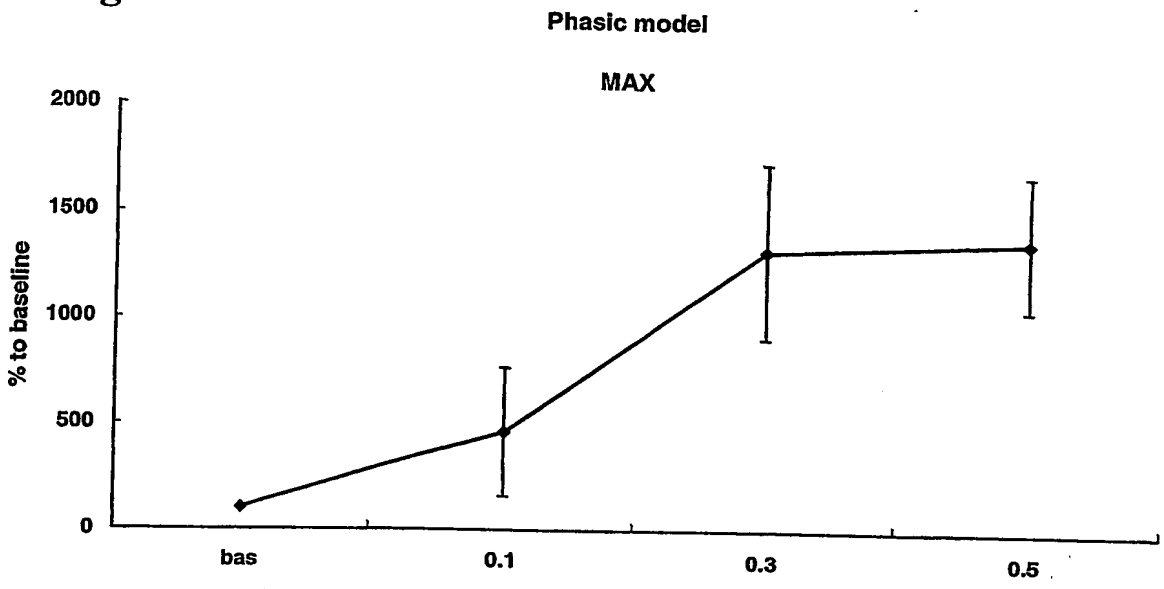


Fig. 10B

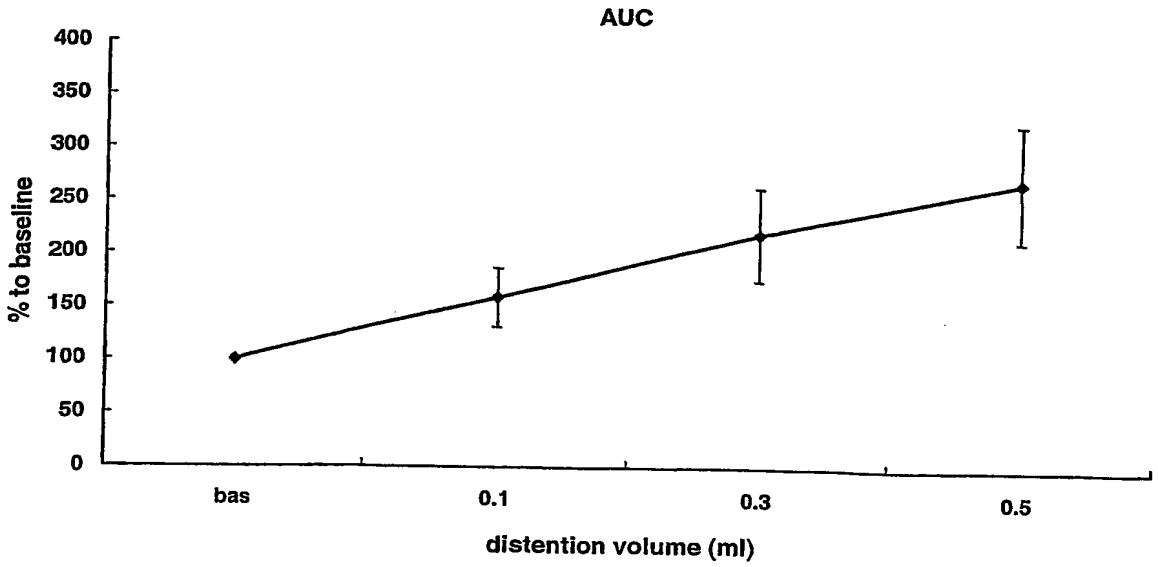


Fig. 11A

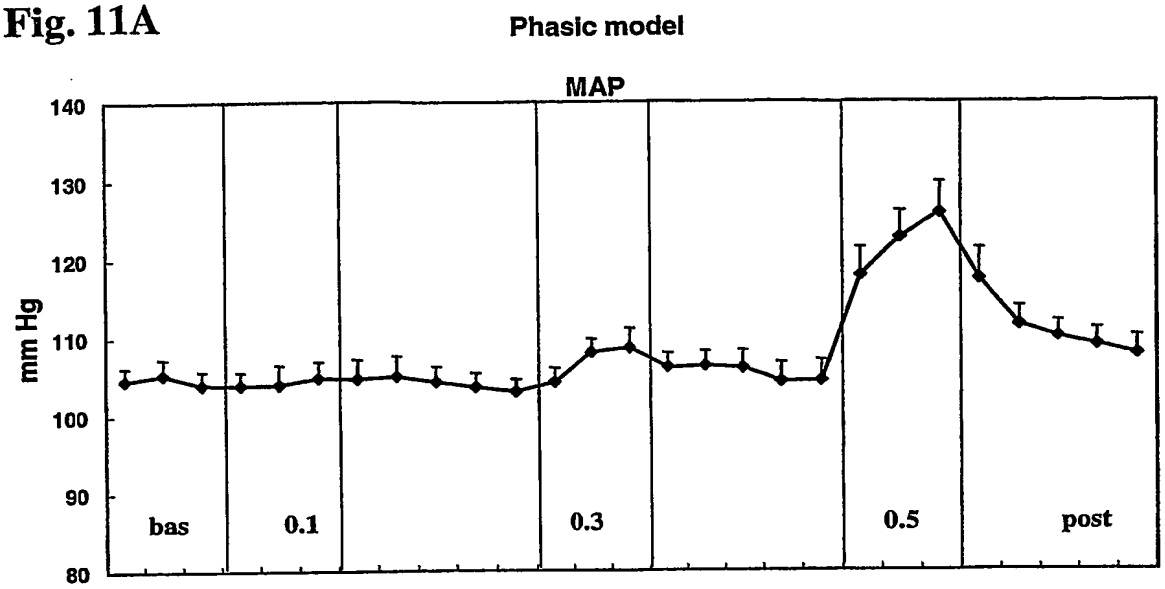


Fig. 11B

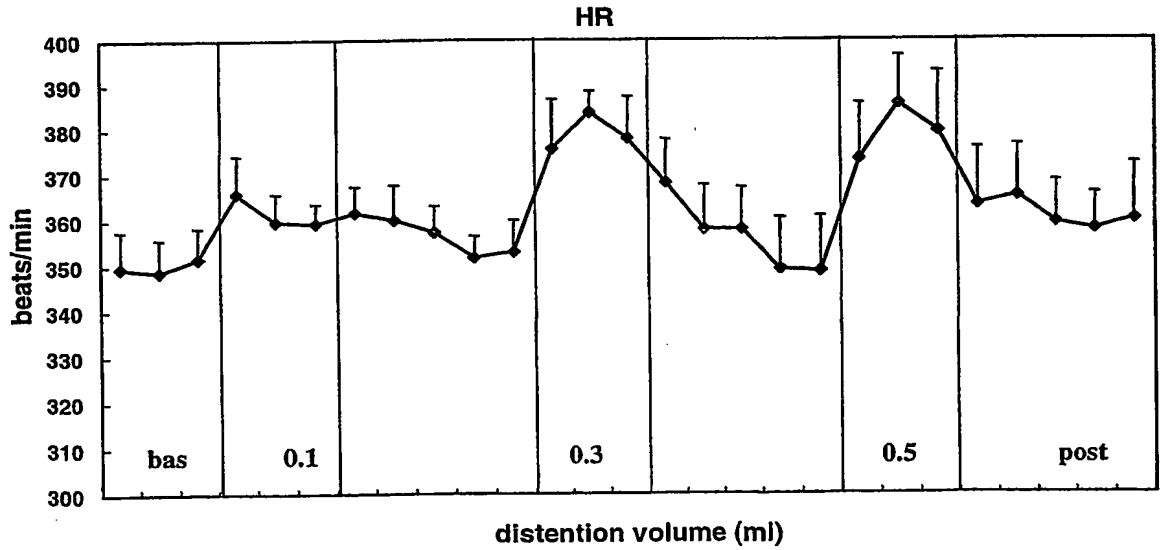


Fig. 12A

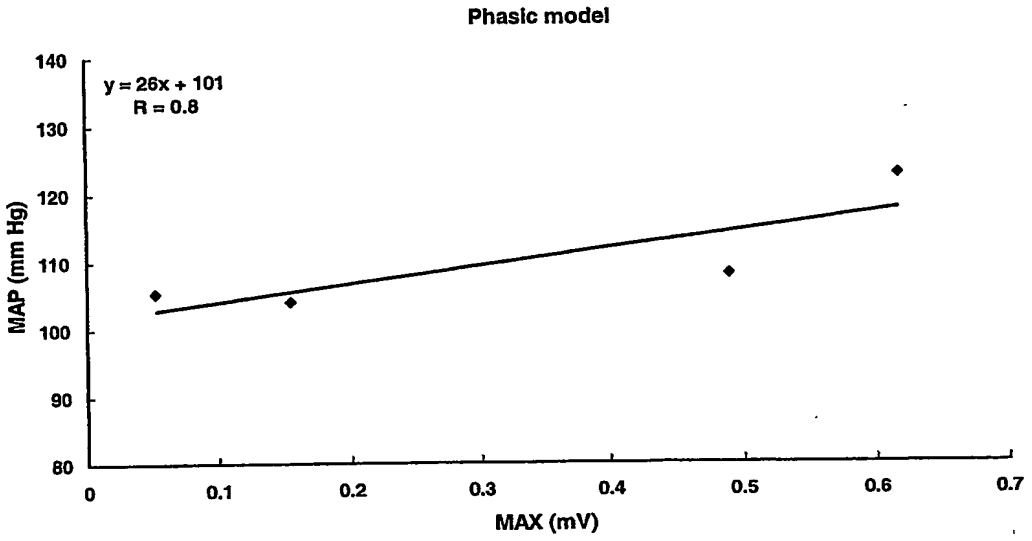


Fig. 12B

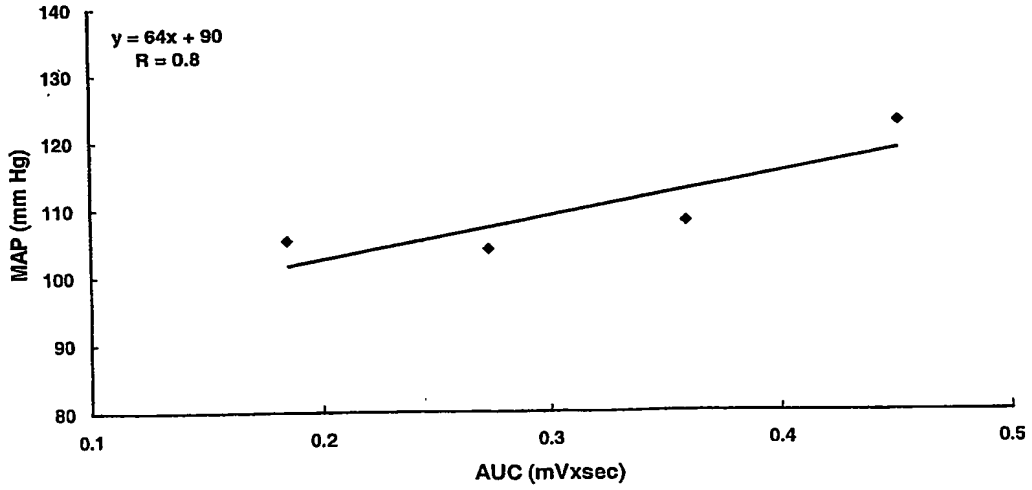


Fig. 13A

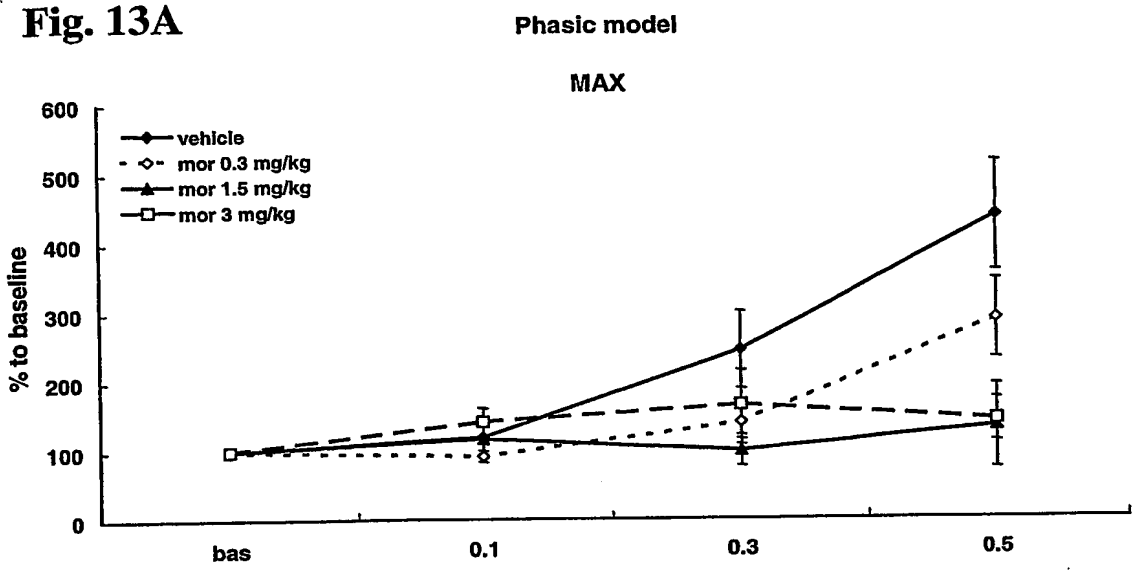


Fig. 13B

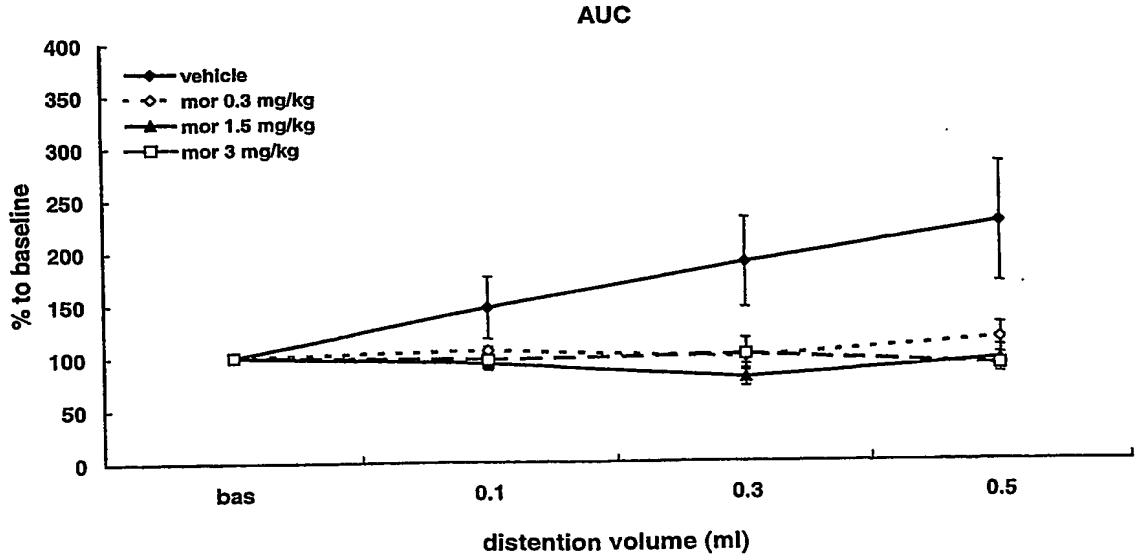
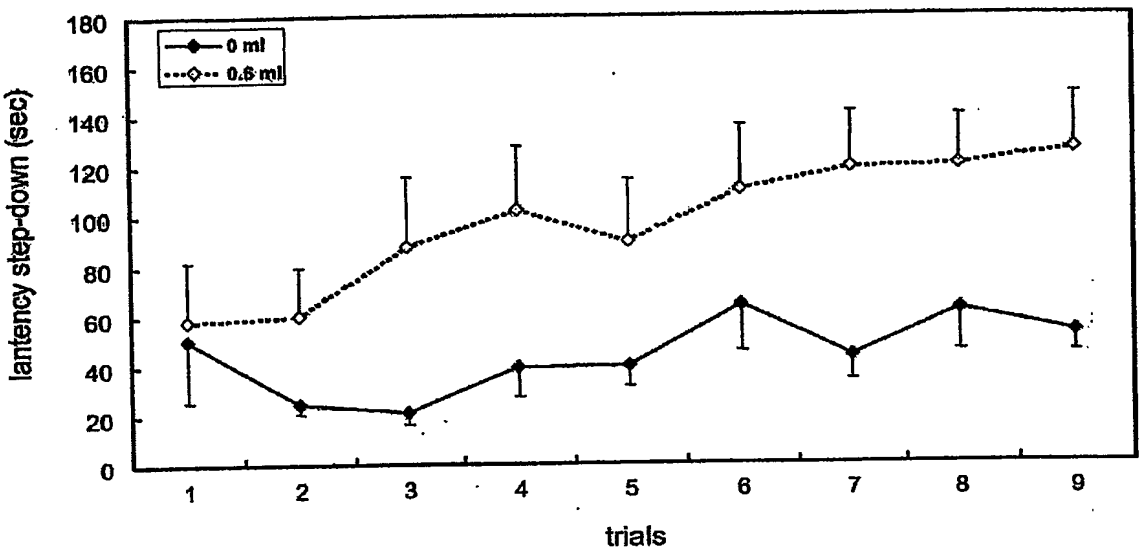


Fig. 14


0 ml
0.6 ml
latency step-down (sec)
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
trials

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